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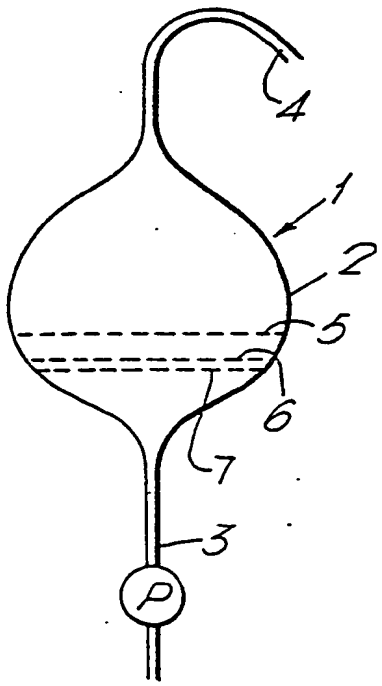
(54) Title: CELL GROWTH

(57) Abstract

A method for the growth of cells in a nutrient medium, which comprises causing the nutrient medium to flow upwardly with decreasing velocity, substantially without turbulent flow, and allowing cells to grow at an equilibrium level in the flow. The method can be conducted in apparatus comprising a vessel (1) of increasing and then decreasing cross-section (greatest at 2) in the direction of flow from a bottom inlet (3) to a top outlet (4). The used medium can be regenerated, if desired.



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CELL GROWTH

FIELD OF THE INVENTION

This invention relates to a method for cell growth, and in particular to the growth of cells in a nutrient medium.

BACKGROUND OF THE INVENTION

It is of course conventional to grow cells in a nutrient medium. The nutrient medium may be stationary, e.g. in a dish, in which case it is tiresome, especially on a large scale, to separate the products of cell growth from the system. Cells may also be grown in a fluent medium, e.g. in order to stimulate cell/nutrient contact and in order to facilitate the replacement of nutrient as it becomes exhausted; however, it is usually then necessary to stir the system, and stirring involves undesirable mechanical attrition.

PRIOR ART

GB-A-1546554 describes maintaining cells, e.g. lymphoblastoid cells, in suspension in a nutrient medium. As the result of introducing oxygen into the bottom of a cylindrical channel containing the medium, the medium flows upwardly at a uniform rate. The medium reaching the top of the channel is passed downwardly, outside the channel, to provide circulatory flow. Accordingly, cells are maintained in suspension by careful choice of oxygen inflow rate.

Examples of documents in which cells are stirred or otherwise moved in a nutrient medium are WO-A-84/03709, in which there is a circulatory flow and an orthogonal flow component caused by the introduction of oxygen; EP-A-0078061, in which the partial pressure of oxygen is changed during the growth phase (the cells must be non-adhering mammalian cells); and WO-A-82/03227, in which laminar flow is maintained in a "movable culture unit".

EP-A-0050562 states as its object a process according to earlier proposals for cell growth, but in the complete absence of any means of cell immobilisation. The disclosure is of circulating a nutrient medium, in sequence, downwardly through two cylindrical vessels, into one of which oxygen and nutrients can be introduced. The other is the "culture vessel", which includes towards its lower end means for separation of tissue material, allowing the downward passage of medium for recirculation.

SUMMARY OF THE INVENTION

A method for the growth of cells in a nutrient medium comprises causing the nutrient medium to flow upwardly with decreasing velocity, substantially without turbulent flow, and allowing cells to grow at an equilibrium level, in the flow. The invention is based upon the principle of flotation, so that cells of one type can grow at a given level in a steady flow, according to the upward velocity of the nutrient medium and the opposing force of gravity, but important advantages are associated with this system.

ADVANTAGES OF THE INVENTION

Firstly, apparatus can be engineered to provide a wide variation of flow velocities which can allow the simultaneous growth of various cells (which can form different strata, according to their sedimentation velocity, in the apparatus). Secondly, cells grow very well in such a system, supported only by the medium, and indeed faster than in a stirred system where they are caused to move. Thirdly, it can easily be arranged that the cells are constantly provided with fresh medium, e.g. if spent medium removed from the top of the apparatus is regenerated or replaced before being passed back up the apparatus. Fourthly, and perhaps most importantly, the products of cell growth, metabolism or lysis are passed

upwardly through the apparatus and can be separated from the medium in a cell-free environment; the products may be, for example, antibodies, other desirable proteins and also dead matter, e.g. from cell walls. Cells may die naturally or may be induced to rupture by the introduction of suitable materials into the flow, e.g. cell wall-destroying enzymes.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention will now be illustrated by way of example only with respect to the accompanying drawings, in which:

Figure 1 is an essentially schematic view of apparatus which can be used in the method of the invention;

Figures 2, 3 and 5 are scale diagrams of three further alternative apparatus which can be used in the invention;

Figures 4 and 6 illustrate systems in which the invention can be operated, the latter in the form of a flow diagram; and

Figure 7 is a schematic representation of a unit which can be used as part of the system illustrated in Fig. 6, and in more detail than Fig. 6.

DETAILED DESCRIPTION OF THE INVENTION

Figs. 1 to 5 show a vessel 1 having a section of gradually increasing and then decreasing diameter, the maximum diameter being shown at 2. There is an inlet 3 at the lower end of the vessel and an outlet 4 at the upper end. The outlet 4 can lead, for example, to means for collection and/or regeneration and/or separation.

Fig. 1 also shows a pump P. In operation, the pump P causes nutrient medium to flow upwardly through the vessel 1. Whatever means is used to cause flow, in any illustrated apparatus, the flow velocity is greatest at the inlet 3 and the outlet 4, and least at the greatest

diameter 2. There is a continuous variation in flow velocity. Cellular material which has been introduced into the vessel finds a certain level, and three stratified bands of cells of different densities are shown at 5, 6 and 7. Products of cellular degradation and metabolism are obtained through the outlet, and the design of the apparatus ensures that there is no turbulent flow below 2, i.e. at the levels of the bands at least. No strata can be formed above the greatest diameter 2, where flow accelerates and removes all material from the vessel 1.

Additional features illustrated in Figs. 2 to 5 are arrows indicating the flow of direction of the medium; a reservoir intake point 8 and a medium collection unit 9 (partially illustrated) in Fig. 2; a reservoir 9 including a Marriott tube 10, silicone tubing 11 and a filling bell 12 in Fig. 4; and three sampling tubes 13 in Fig. 5. The design of the apparatus illustrated in Fig. 4 is adapted to the use of higher flow rates than can be used in other illustrated apparatus, without turbulence. Relatively high flow rates may be useful when, as is the case for lymphoblastoid cells, clumps of cells can form which can be maintained in suspension (obviously relatively nearer the bottom of the vessel than individual cells). The apparatus of Fig. 4 provides a trap to prevent back-flow of adherent cultured cells into the reservoir. The tube from the culture vessel to the filling bell prevents the accumulation of bubbles of gas absorbed through the silicon tubing.

Fig. 6 shows a culture vessel 1 with sampling tubes 13. Nutrient medium is passed through the vessel 1 upwardly, in the direction of the arrows, on operation of the pump P. Medium is taken from, and returned to, a sealed mixing reservoir 14, e.g. 1 litre in volume. The mixing reservoir 14, schematically represented in Fig. 6,

is illustrated in more detail in Fig. 7; it includes a fresh medium inlet 15, a medium collection outlet 16, a culture vessel medium outlet 17, a spent culture vessel medium inlet 18, an air/CO_2 inlet 19, an air outlet 20, a sample collection tube 21, and a pH probe 22. Stirring can be effected by means of a magnetic stirrer 23 and magnetic follower 24 (shown only in Fig. 7).

Fig. 6 also shows a fresh medium reservoir 9, a pH meter/controller 25 and, electrically connected thereto, a pump 26 which introduces fresh medium, when necessary, and removes medium for collection (see also Example 4). The use of apparatus as illustrated in Figs. 6 and 7 allows maximum economy in terms of medium usage, and also the recirculation of products of cell metabolism, lysis, etc. which may beneficially aid cell growth.

The following Examples illustrate the invention.

Example 1

An apparatus was constructed in borosilicate glass, with the properties and dimensions shown in Fig. 2. The glass was cleaned with chromic acid, and then attached by silicon tubing to a reservoir and filling bell, as shown in Fig. 3. The entire apparatus was flushed through with a concentrated solution of citric acid and then with at least 10 changes of double glass-distilled water. The entire apparatus was sterilised by autoclaving.

Growth medium (RPMI 1640 with 10% selected foetal calf serum) was placed in the reservoir, and run through the apparatus. The flow rate was controlled by a variable tubing clamp and measured by collecting medium in a measuring cylinder below the filling bell for measured times. Cells (see Examples 2 and 3) were introduced into the tubing with an hypodermic needle and syringe. At a flow rate of 0.5 ml/min, the cells formed horizontal bands in the apparatus. The entire apparatus

was mounted in a frame, and placed in a carbon dioxide incubator at 37 C, pH 100%, 95% carbon dioxide. The medium reservoir was replenished with medium as necessary, and the effluent medium was collected in sterile glass containers.

The medium collected from the filling bell was tested for immunoglobulin and antibody activity. The immunoglobulin levels were about 1 to 5 µg/ml, and antibody activity was detectable by a sensitive enzyme immuno-assay.

Example 2

Human lymphoblastoid cells were introduced into apparatus as in Example 1.

At a flow rate of 0.5 ml/min, a band of single cells could be seen several centimetres from the bottom of the apparatus. Clumps of cells, of various sizes, were present below the single cells, the larger clumps being nearer the bottom. A large "mass" of cells or clumps was present in the tube at the bottom of the apparatus; as the flow rate was increased each "band" of cells and clumps moved upward in the apparatus, when it became clear that the large "mass" at the bottom consisted of larger clumps of cells.

The human lymphoblastoid cells produced antibody against Pseudomonas aeruginosa serotype 11.

The culture was maintained for 35 days. During this time the number of cells appeared to increase (the bands became denser, and the lowermost aggregate of clumps became larger). The concentration of human immunoglobulin in the collected medium remained at between 1 and 5 µg/ml.

After 35 days, the culture became contaminated with bacteria.

Example 3

Hybrid cells, derived from the fusion of mouse myeloma NS0 and mouse spleen cells producing antibody, were introduced into apparatus as in Example 1.

The cells did not aggregate. They formed a band less than one centimetre deep, several centimetres above the bottom of the apparatus.

The culture was maintained for 28 days. During this time the density of the band varied. The concentration of mouse immunoglobulin in the collected medium remained at between 3 and 5 µg/ml.

After 28 days, the culture became contaminated with bacteria.

Example 4

The culture vessel illustrated in Fig. 5 was incorporated into a fluid flow circuit, as illustrated in Fig. 6. The culture vessel was constructed from a two litre volumetric flask. Medium was pumped into the culture at a controlled rate using a Watson Marlow pump, type MHRE 200. The medium as pumped from, and then back into, a reservoir, which was a Gallenkamp Culture Vessel Module (catalogue number FBL-195-010D), with magnetic stirrer (FBL-280) and magnetic follower, rotating at 60 rpm. The reservoir was supplied with 10% carbon dioxide in air at 200 ml/min, as illustrated in more detail in Fig. 7. The ports in the top plate of the reservoir carried a filtered air outlet, with reflux air condenser, a pH probe attached to a pH meter (FBL-734-010D), a medium inlet tube, a medium outlet tube, a sampling tube, and a carbon dioxide and air inlet tube. All glass tubing was light-wall borosilicate glass, outside diameter 4 mm, wall thickness 0.8 mm. All flexible tubing was medical grade silicone rubber, internal diameter 3 mm, outside diameter 6 mm; before use it was boiled in double-distilled water for one hour and stored

in distilled water. The pH meter was modified (by Gallenkamp) to drive a two-channel peristaltic pump for the medium inlet and the medium outlet. As the pH dropped to 7.0, fresh medium was introduced and used medium removed, to restore the pH to 7.3. The assembled apparatus and tubing were autoclaved at 100 kPa (15 psi) for 15 minutes.

The culture vessel was maintained at 37 C and the reservoir was maintained at 4 C. The two were connected by coils of borosilicate glass tubing; the temperature of the medium was raised or lowered by passage from one to the other. The apparatus and tubing were filled with RPMI1640 medium supplemented with 10% foetal calf serum, L-glutamine and sodium pyruvate, but without antibiotics.

Hybrid cells, derived from the fusion of a mouse myeloma cell with a mouse splenic B lymphocyte, were introduced into the culture vessel. They formed a band, the height of which was dependent on the flow rate of the medium.

During the culture period of 21 days, the concentration of hybrid cells in the band rose from 3 x 10^7 per ml to 8 x 10^{12} per ml. The band of cells was clearly visible. Mouse immunoglobulin, with specific antibody, was harvested from the apparatus over a period of 14 days. At the end of this time, a contaminated batch of medium caused bacterial contamination of the entire culture.

CLAIMS

1. A method for the growth of cells in a nutrient medium, which comprises causing the nutrient medium to flow upwardly with decreasing velocity, substantially without turbulent flow, and allowing cells to grow at an equilibrium level in the flow.

2. A method according to claim 1, which comprises introducing medium at the lower end of a vessel having increasing and then decreasing cross-section, in the direction of flow, and removing medium together with any products of cell metabolism from the upper end of the vessel.

3. A method according to claim 1 or claim 2, in which the medium is regenerated after use and recirculated.

4. Apparatus which comprises:

a vessel through which a nutrient medium can be passed upwardly, having increasing and then decreasing cross-section, in the direction of flow;

means for regenerating the medium; and

means for circulating the medium through the vessel and the regeneration means.

5. Apparatus according to claim 4, which additionally comprises:

means for monitoring the condition of the medium, in response to which fresh medium can be introduced.

Fig.1.

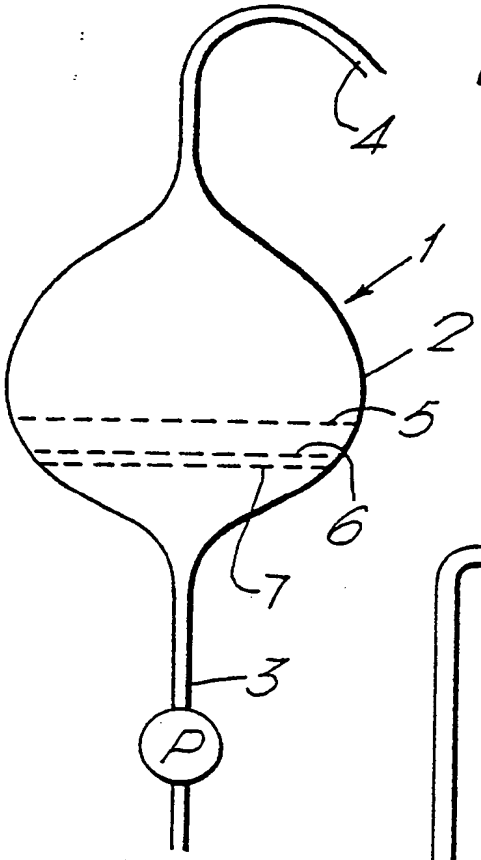


Fig.2.

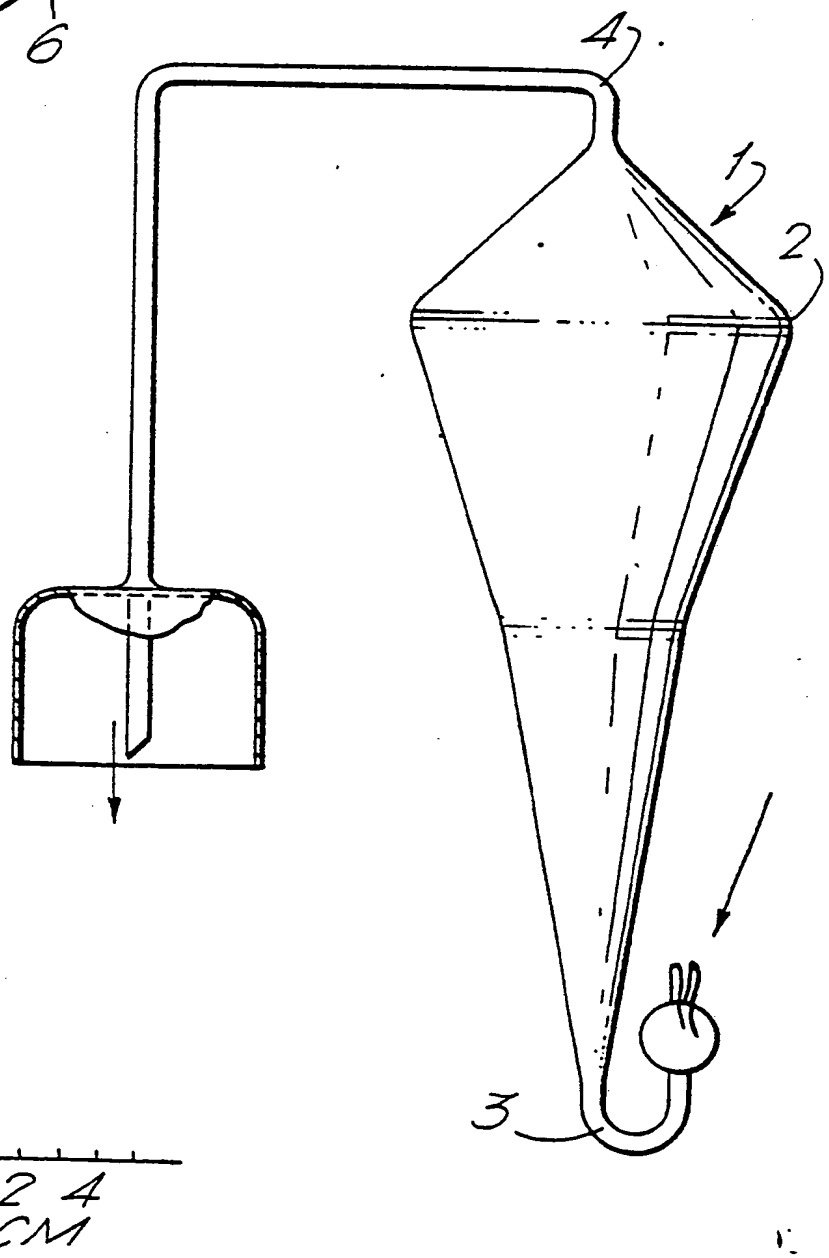


Fig.3.

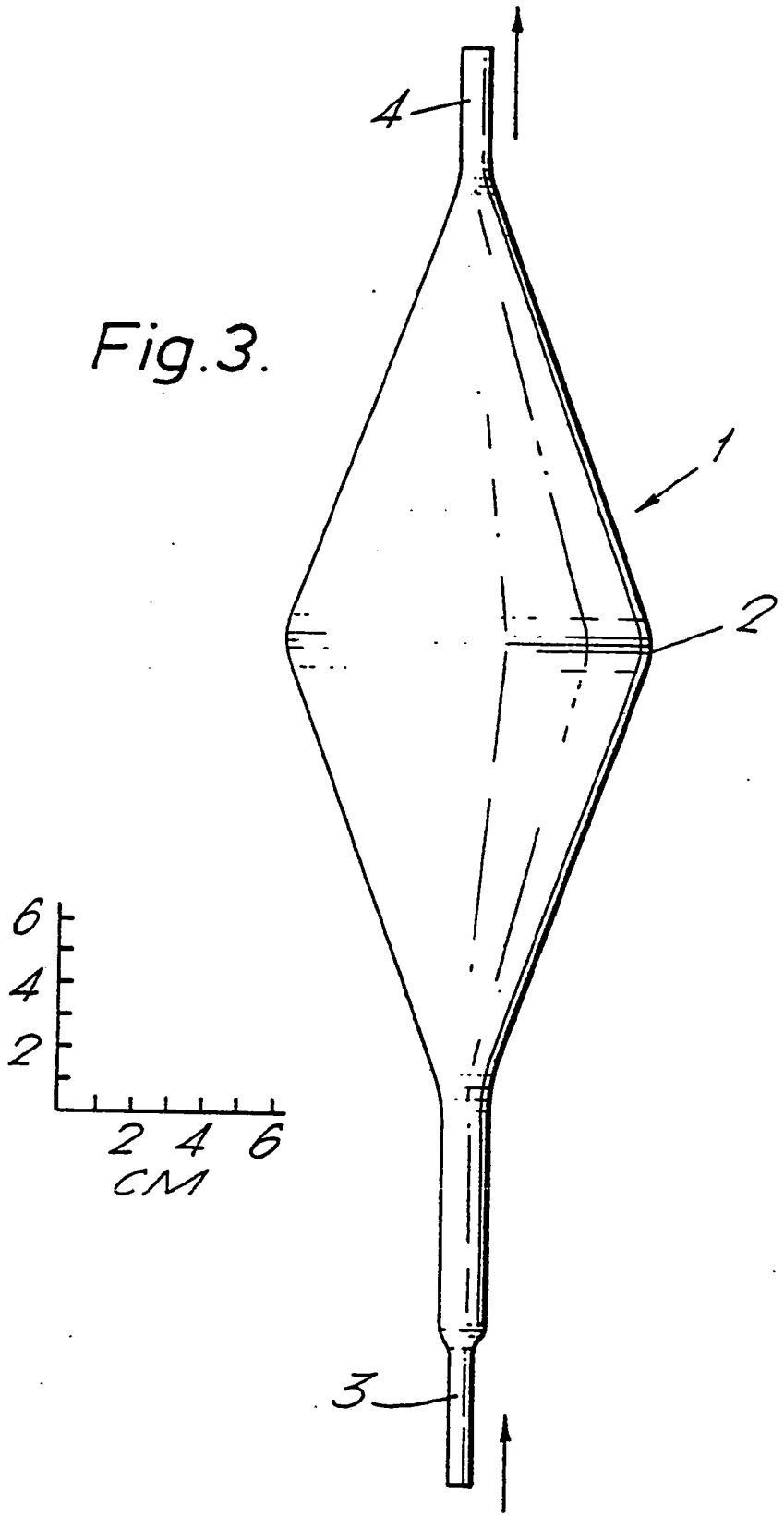
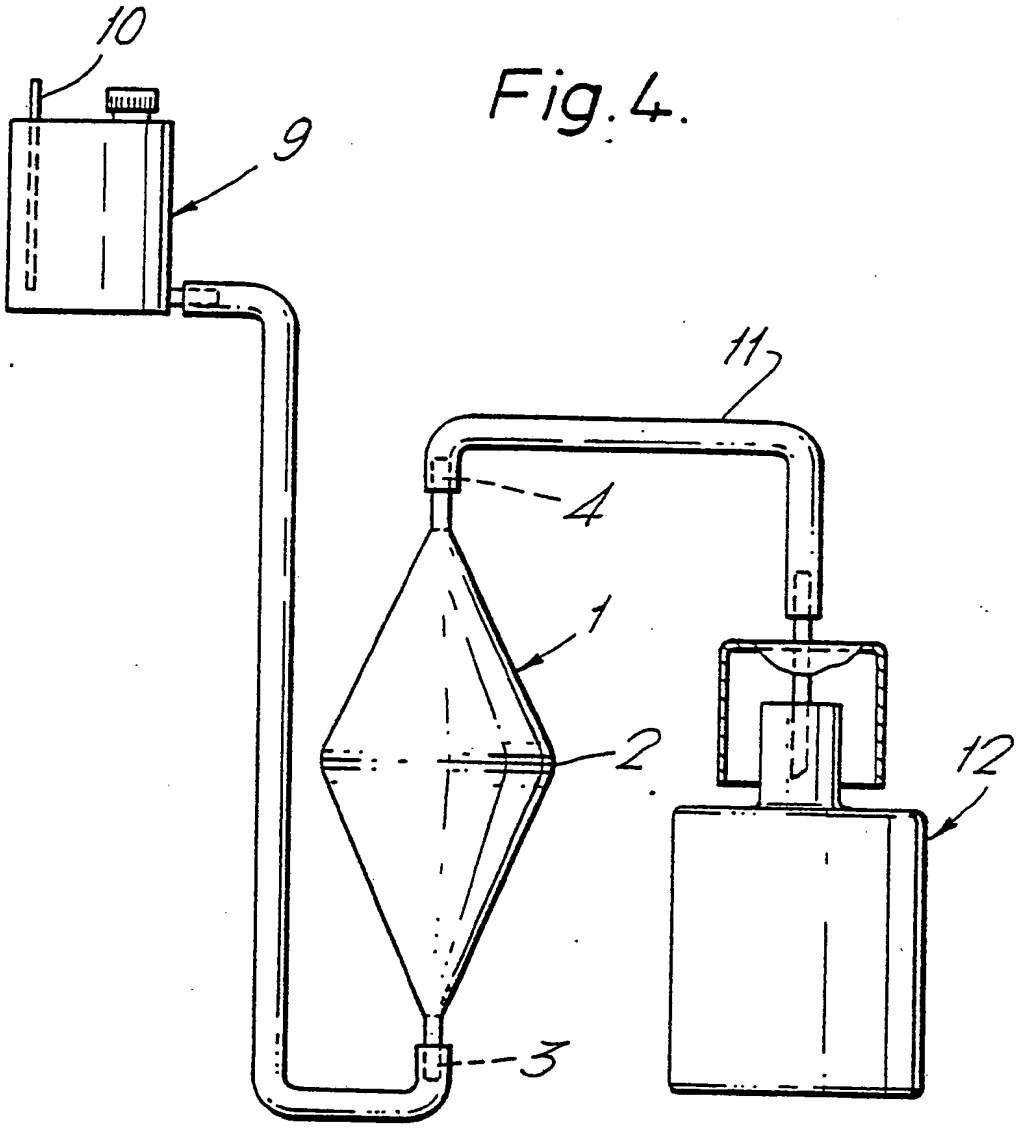


Fig. 4.



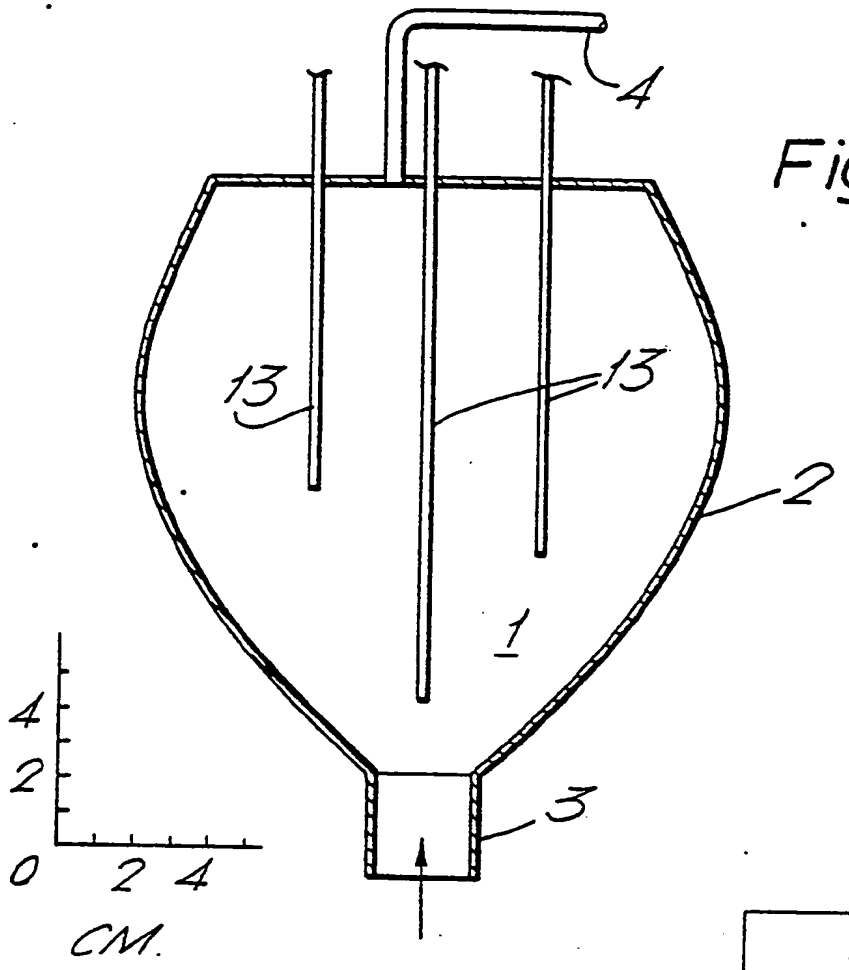


Fig.5.

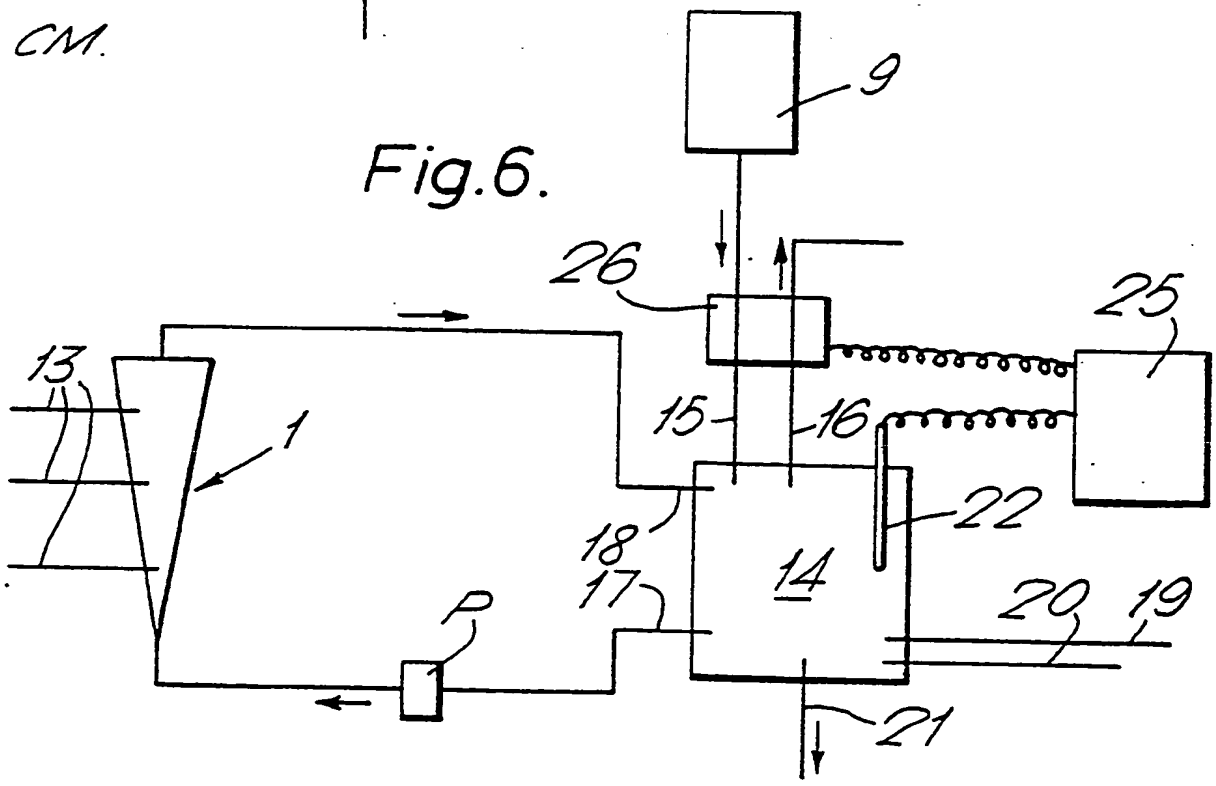
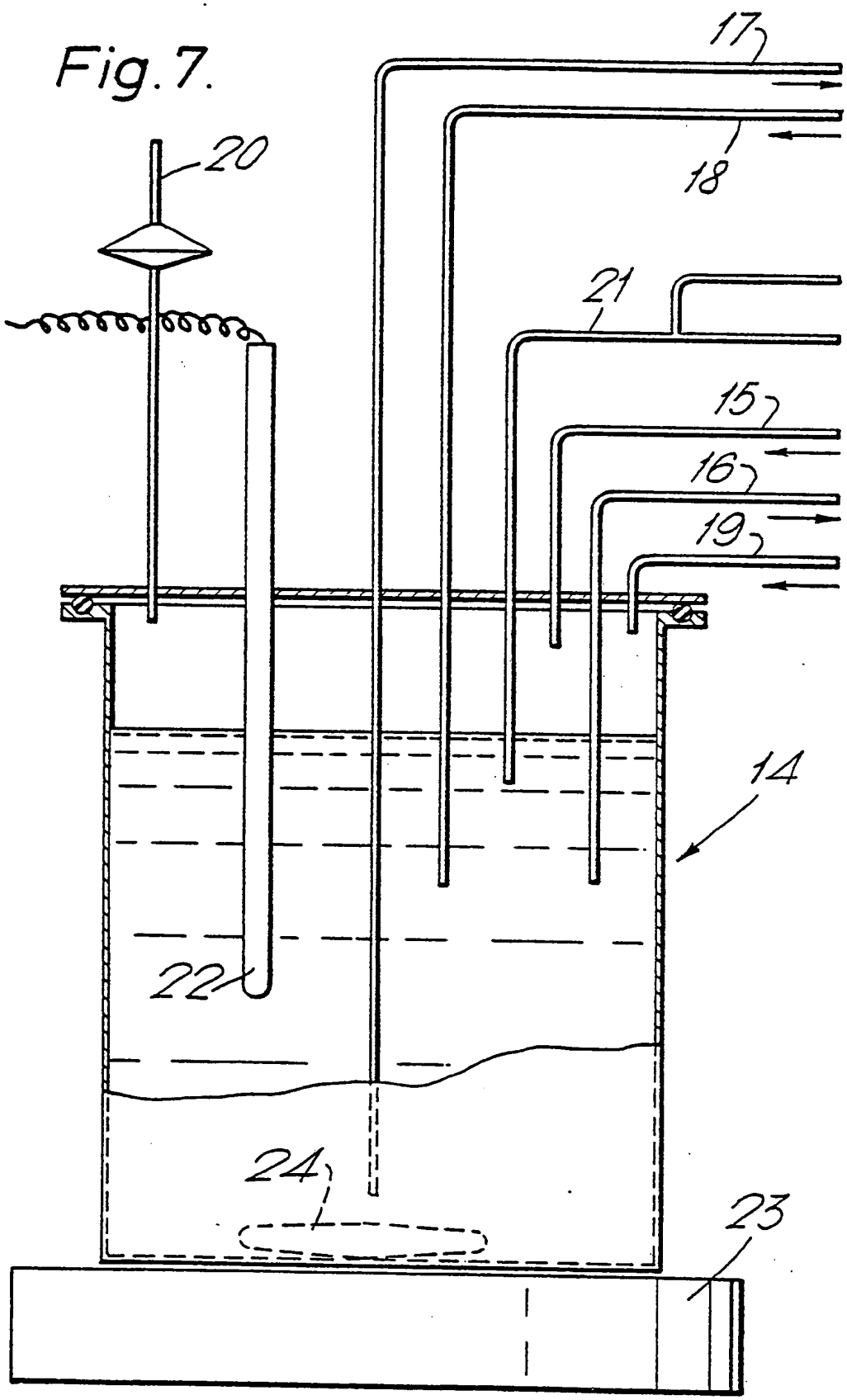


Fig.6.

Fig.7.



INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 85/00307

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[4]: C 12 M 3/02

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[4]	C 12 M

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	US, A, 3591460 (D.F. SMITH) 6 July 1971, see figures; claims; column 2, lines 15-67	1-5

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
10th October 1985	01 NOV 1985
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	G.J.M. Kruydenberg

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ANNEX TO THE INTERNATIONAL SEARCH REPORT ON

INTERNATIONAL APPLICATION NO. PCT/GB 85/00307 (SA 10086)

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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